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SPEEDTOOLS DNA EXTRACTION KIT

Designed for the rapid isolation of highly pure genomic DNA from cultured cells, whole blood, serum, plasma, or other body fluids

Instructions for Use

(Ref. 21.130M/1/2)

PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.

1. BASIC PRINCIPLE

SPEEDTOOLS DNA EXTRACTION KIT is designed for the rapid isolation of highly pure genomic DNA from:

- cultured cells
- whole blood
- serum
- plasma
- other body fluids

Lysis of sample is achieved by incubation of sample in a solution containing chaotropic ions in the presence of proteinase K. Addition of molecular grade ethanol to the lysate create the appropriate conditions for binding of DNA to the silica membrane of the column. This binding process is reversible and specific to nucleic acids. Washing steps efficiently remove contaminations and pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2. KIT CONTENTS

SPEEDTOOLS DNA EXTRACTION KIT			
Reagent	**10 Preps Ref. 21.130M**	**50 Preps Ref.21.131**	**250 Preps Ref.21.132**
Buffer BB3	3 ml	15 ml	5 x 15 ml
Buffer BB5 (concentrate)	3 ml	12 ml	5 x 12 ml
Buffer BBW	6 ml	30 ml	5 x 30 ml
Buffer BBE	3 ml	13 ml	5 x 13 ml
Proteinase K (lyophilised)	6 mg	30 mg	5 x 30 mg
Proteinase Buffer (PB)	400 µl	1.8 ml	5 x 1.8 ml
DNA Binding Columns (plus Collection Tubes)	1 x 10	50	5 x 50
Collection Tubes	1 x 20	100	5 x 100

3. INTENDED USE

With the **SPEEDTOOLS DNA** method, genomic DNA is prepared from cultured cells, whole blood, serum, plasma, or other body fluids. It is also possible to purify viral DNA from blood samples. As viral DNA co-purifies with cellular DNA, we recommend usage of cell-free sample (serum or plasma) to prepare pure viral DNA.

Blood treated either with EDTA, citrate, or heparin can be used. If leukocyte rich materials like buffy coat are used, apply smaller volumes and dilute the samples with sterile PBS (dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO4, and 0.24 g KH_2PO4 in 800 ml H_2O. Adjust pH to 7.4 with HCl. Add H_2O to 1 Liter).

The kits allow isolation of highly pure genomic DNA with an A260/280-ratio between 1.60 and 1.90 and a typical concentration of 40 – 60 ng per µl.

The obtained DNA is ready to use for subsequent reactions like PCR, Southern blotting, or any kind of enzymatic reactions.

Table 1: General Characteristics of the Kit	
	Sample
Sample size	Up to 200 µL
Average Yield	4-6 µg
Elution Volume	100 µl
Binding Capacity	60 µg
Time / Prep	30 min
Spin Column Type	mini

4. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

NOTE: Buffers BB3 and BBW contain guanidine hydrochloride therefore wear gloves and goggles.

All kit components can be stored at room temperature (18-25°C) through the expiration date printed on the packaging label.

Before starting any protocol with **SPEEDTOOLS DNA EXTRACTION KIT** prepare the following reagents:

I. **Proteinase K:**

- ✓ **10 preps format:** Add **260 µL** of Proteinase Buffer to dissolve lyophilized Proteinase K.
- ✓ **50 preps format:** Add **1.35 mL** of Proteinase Buffer to dissolve lyophilized Proteinase K.

The Proteinase K Solution is stable at –20°C for at least 6 months.

II. Buffer BB5:

- ✓ **10 preps format:** Add **24 mL of molecular grade ethanol** (96-100%) to buffer BB5 concentrate. Mark the label of the bottle to indicate that ethanol was added.
- ✓ **50 preps format:** Add **48 mL of molecular grade ethanol** (96-100%) to buffer BB5 concentrate. Mark the label of the bottle to indicate that ethanol was added.

Wash Buffer BB5 can be stored at room temperature (18-25°C) for at least 12 months.

Upon storage, especially at low temperatures, a white precipitate may form in Buffer BB3. Dissolve such precipitates by incubation of the bottle at 50-70°C before use.

5. DNA ELUTION PROCEDURES

The *Standard Protocol* described in Section 6 has a recovery rate about 70-90%. It is possible to adapt the elution method and volume of elution to the subsequent application of interest. In addition to the *Standard Protocol* there are several modifications possible to increase yield, concentration, and convenience.

A) High yield: Perform two elution steps with the volume indicated in the individual protocol. About 90-100% of bound nucleic acid can be eluted.

B) High concentration: Perform one elution step with 60% of the volume indicated in the individual protocol. Concentration of DNA will be approximately 30% higher than with standard elution. The yield of eluted DNA will be about 80%.

C) High yield and high concentration: Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. About 85-100% of bound nucleic acid is eluted in the standard elution volume (100 µl) at a high concentration.

D) Elution at 70 ºC: For certain sample, heating the elution buffer to 70 °C increases the DNA yield.

Elution may also be performed with Tris-EDTA buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and/or multi use storage at 4°C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes (depending on the final concentration) with certain downstream applications.

NOTE: Elution Buffer BBE (5 mM Tris/HCl, pH 8.5) provided with the kit does not contain EDTA.

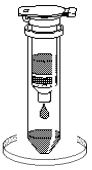
For optimal performance of isolated DNA in downstream applications we recommend elution with the supplied elution Buffer BBE and storage, especially long term, at -20°C. Several freeze-thaw cycles will not interfere with most downstream applications. Possible exceptions are detection of trace amounts of DNA or long-range PCR (e.g. > 10 kb). Multiple freeze-thaw cycles, or storing DNA at 4°C or room temperature, my influence detection sensitivities or reaction efficiencies due to DNA shearing or adsorption to surfaces.

6. INSTRUCTION FOR USE

A. Standard Protocol

Before starting the preparation:

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set incubators or water baths to 70 and 56ºC.
- Before elution, preheat Elution Buffer BBE to 70ºC.

STEP	DESCRIPTION		
1	**SAMPLE LYSIS** • Pipette **25 µl Proteinase K** and up to **200 µl of sample** (equilibrated to room temperature) into 1.5 ml microcentrifuge tube. *For sample volumes less than **200 µl blood; body fluid sample; etc**, add PBS to adjust the volume to 200 µl. If purifying DNA viruses, we recommend starting with 200 µl serum or plasma. If cultured cells are used, resuspend up to 5 × 10^6 cells in a final volume of 200 µl PBS.* • Add **200 µ**l of lysis **Buffer BB3** to samples and **vortex** the mixture vigorously (10-20 s). Incubate samples at **70°C** for **10 - 15 min**. *The lysate should become brownish during incubation with buffer BB3. Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood sample*s.		**200 µl SAMPLE + 25 µl PROTEINASE K + 200 µl BUFFER BB3** **mix** 70°C 10-15 min
2	**ADJUST DNA BINDING CONDITIONS** Add **210 µl of molecular grade ethanol** (96-100%) to each sample and **vortex** again.		**+ 210 µl MOLECULAR GRADE ETHANOL mix**
3	**BIND DNA** For each preparation, take one **column** placed in a 2 ml Collection tube and **load the sample**. Centrifuge **1 min** at **11,000 × *g*.** If the samples are not drawn through the matrix completely, repeat the centrifugation at higher *g*-force (< 15,000 × *g*). **Discard the flow-through** and place the column back into the Collection tube.		**Load lysate into a column** 1 min, 11,000 × g

4	**WASH SILICA MEMBRANE** **1st Wash** Add **500 µl** Buffer BBW. Centrifuge 1 min at 11,000 x *g*. Discard flow-through and place the column back into the Collection tube. **2nd Wash** Add **600 µl** Buffer BB5. Centrifuge 1 min at 11,000 x *g*. Discard collection tube with flow-through.		**+** **500 µl** **BUFFER BBW** *1 min, 11,000 × g* **+** **600 µl** **BUFFER** **BB5** *1 min, 11,000 × g*
5	**DRY SILICA MEMBRANE** Place the column into 1.5 ml microcentrifuge tube and centrifuges 1 min at 11,000 x *g*. Residual ethanol is removed during this step.		*1 min, 11,000 × g*
6	**ELUTE HIGHLY PURE DNA** Place the column in a new 1.5 ml microcentrifuge tube and add 100 µl prewarmed elution Buffer BBE (70°C). Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x *g*. The eluate contains your pure DNA sample. ***For alternative elution procedures see section 5.***		**+** **100 µl** **BUFFER BBE** **(70ºC)** **Incubate** **1 min** *1 min, 11,000 × g*

NOTE

For viscous sputum samples is necessary to pre-treat the samples according to the following scheme before using the Speedtools DNA Extraction Kit:

1. Add 1 volume NALC solution to 1 equal volume sample; incubate at room temperature for 20 min.
NALC Solution:

1.45 % sodium citrate
2 % sodium hydroxide
0.5 % N-acetyl-L-cysteine

2. Centrifuge sample at 3,500rpm for 20 minutes.

3. Remove and discard supernatant.

4. Resuspend pellet in 1ml 1% PBS.

5. Use 200 µl for Speedtools DNA extraction Kit.

B. Protocol for extraction and purification of plasma CMV DNA

Before starting the preparation:

- Check if Buffer BB5 and Proteinase K were prepared according to Section 4.
- Set an incubator or water bath to 70ºC
- Before elution, preheat Elution Buffer BBE to 70ºC.

STEP	DESCRIPTION		
1	**SAMPLE LYSIS** • Pipette **25 µl Proteinase K** and up to **250 µl of plasma** (equilibrated to room temperature) into 1.5 ml microcentrifuge tube. • Add **200 µ**l of lysis **Buffer BB3** to the samples and **vortex** vigorously (10-20 s). Incubate samples at **70°C** for **10 - 15 min**.		**250 µl PLASMA + 25 µl PROTEINASE K + 200 µl BUFFER BB3 mix** 70°C 10-15 min
2-5	***Proceed with Step 2 of the Standard Protocol.*** At Step 3 in order to load the complete lysate perform two loading steps.		
6	**ELUTION AND CONCENTRATION OF CYTOMEGALOVIRUS DNA** Place the column in a new 1.5 ml microcentrifuge tube. Add **40 µl** pre-warmed elution Buffer BBE (70°C). Dispense buffer directly onto the silica membrane. Incubate at room temperature for 3 min. Centrifuge 1 min at 11,000 x *g*. The eluate contains your pure DNA sample.		**+ 40 µl BUFFER BBE (70ºC)** **Incubate 3 min** *1 min, 11,000 × g*

7. TROUBLESHOOTING

Problem	Possible cause and suggestions
No yield or poor DNA yield	***Low concentration of leukocytes in sample*** • Prepare buffy coat from the blood sample: Centrifuge whole blood at room temperature (3,300 × *g*; 10 min). Three different layers will be visible after centrifugation. Leukocytes are concentrated in the intermediate layer (buffy coat). ***Incomplete cell lysis*** • Sample not thoroughly mixed with lysis buffer / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer. • Proteinase K digestion not optimal. Never add Proteinase K directly to lysis buffer. Incubate for 15 - 20 min at 70°C. ***Reagents not applied properly*** • Prepare Buffer BB5 and Proteinase K solution according to instructions (section 4). Add molecular grade ethanol to lysates before loading them on columns. ***Suboptimal elution of DNA from the column*** • Preheat Buffer BBE to 70°C before elution. Apply Buffer BBE directly onto the center of the silica membrane. • Elution efficiencies decrease dramatically if elution is performed with buffers of pH < 7.0. Use slightly alkaline elution buffer like Buffer BBE (pH 8.5). • Mix vigorously once during the 70°C incubation step especially when working with old or clotted blood samples.
Suboptimal performance of genomic DNA in enzymatic reactions	***Carryover of ethanol*** • Be sure to remove all of ethanolic Buffer BB5 before eluting the DNA. If the level of BB5 after the second wash has reached the column outlet for any reason, discard flow-through, place the column back into the collection tube, and centrifuge again. ***Contamination of DNA with inhibitory substances*** • If DNA has been eluted with Tris/EDTA buffer (TE), make sure that EDTA does not interfere with downstream applications or re-purify DNA and elute in BBE buffer. • If preparing DNA from older or clotted blood samples, extend Proteinase K incubation to 30 min and vortex once or twice during this step. • If the A260/280-ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume of Buffer BB3 plus 1 volume molecular grade ethanol to the eluate, load on column, and proceed with step 3 of the corresponding protocol.

Problem	Possible cause and suggestions
Poor DNA quality	***Reagents not applied properly*** • Prepare Buffer BB5 and Proteinase K solution according to instructions (section 4). Add molecular grade ethanol to lysates and mix before loading them on columns. ***Incomplete cell lysis*** • Sample not thoroughly mixed with lysis buffer / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer. • Proteinase K digestion not optimal. Never add Proteinase K directly to lysis buffer. Incubate for 15 - 20 min at 70°C. ***RNA in sample*** • If DNA free of RNA is desired, add 20 µl of an RNase A solution (20 mg/ml) before addition of lysis buffer. ***Old or clotted blood samples processed*** • For isolation of DNA from older or clotted blood samples, we recommend extension of Proteinase K incubation to 30 min and vortexing several times during this step.

8. ORDERING INFORMATION

SPEEDTOOLS KIT	10 PREPS	50 PREPS	250 PREPS
SPEEDTOOLS **DNA EXTRACTION** KIT	Ref. 21.130M	Ref. 21.131	Ref. 21.132
SPEEDTOOLS **TISSUE DNA EXTRACTION** KIT	Ref. 21.135M	Ref. 21.136	Ref. 21.137
SPEEDTOOLS **RNA VIRUS EXTRACTION** KIT	Ref. 21.140M	Ref. 21.141	Ref. 21.142
SPEEDTOOLS **FOOD DNA EXTRACTION** KIT	Ref. 21.175M	Ref. 21.176	Ref. 21.177
SPEEDTOOLS **PLANT DNA EXTRACTION** KIT	Ref. 21.170M	Ref. 21.171	Ref. 21.172
SPEEDTOOLS **TOTAL RNA EXTRACTION** KIT	Ref. 21.210M	Ref. 21.211	Ref. 21.212
SPEEDTOOLS **PCR CLEAN-UP** KIT	Ref. 21.200M	Ref. 21.201	Ref. 21.202
SPEEDTOOLS **PLASMID DNA PURIFICATION** KIT	Ref. 21.220M	Ref. 21.221	Ref. 21.222

9. PRODUCT USE RESTRICTION AND WARRANTY

1. Product for research purposes and *in vitro uses* only.
2. No claim or representation is intended for its use to identify any specific microorganism or for clinical use (diagnostic, prognosis, therapeutic, or blood bank).
3. It is rather the responsibility of the user to verify the use of the kit for a specific application range as the performance characteristic of this kit has not been verified for a specific microorganism.
4. The kit includes documentation stating specifications and other technical information. Follow the instructions in order to obtain highly pure genomic DNA.
5. BIOTOOLS warrants to meet the stated specifications of the kit. Any product not fulfilling the specifications included in the product sheet will be replaced.
6. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.
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